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Ameliorative Effect of*Moringaoleifera* Leaves Extract on the Intracranial Auditory Relay Centre (Inferior Colliculus) of Male Albino Wistar Rats with Quinine Induced Toxicity

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Abstract :Ethno-pharmacological use of *Moringaoleifera* (MO) leaf is supported by the reported presence of rich antioxidants and phytochemicals. The effect of administration of quinine and *Moringaoleifera* leaf ethanolic extract was investigated using thirty-five (35) albino Wistar rats weighing between 180 – 200 grams. The rats were divided into seven groups with five animals per group. Group 1 served as control while Groups 2 and 5 received 10 mg/kg and 20 mg/kg of quinine respectively 8 hourly for 7 days. Groups 3 received 10 mg/kg of quinine alongside 250 mg/kg of MO extract for 7 days while Group 6 received 20 mg/kg of quinine concomitant with 500 mg/kg of MO leaf extract for 7 days. In Groups 4 and 7, 10 mg/kg and 20 mg/kg of quinine was administered concomitantly with 250 mg/kg and 500 mg/kg respectively for 7 days after which quinine was withdrawn and MO extract continued for another 7 days. Histological evaluation of the inferior colliculus of the brain sections revealed normal structural arrangement in Group 1. Groups 2 and 5 showed dose dependent neurodegenerative features such as neuronal cell degeneration, with reduced neuronal density. Normal neuronal cell and increased neuronal density were observed in rats of Group 3 though slight degeneration of structural features which was less severe was seen in Group 6 when compared to Group 5. Normal neuronal cell, and neuronal density was observed in Group 4 and 7 following further administration of MO extract for another 7 days. The study has shown that *Moringaoleifera* leaf extract has a moderate neuro-protective effect against quinine-induced neurotoxicity in albino Wistar rats.

Keywords *-Moringaoleifera, Quinine, Neurotoxicity, Neuroprotection, Inferior Colliculus*

I. INTRODUCTION

The central nervous system and the peripheral nerves are rich both in unsaturated fatty acids and iron [1]. The high lipid content of the nervous tissue coupled with its metabolic activity is particularly susceptible to oxidative damage. There is substantial evidence that oxidative stress is a causative factor in the pathogenesis of major neurodegenerative diseases [2]. Although limited information is available with the role of oxidative stress in tissue toxicity during administration of quinine [3], the pharmacological therapy currently used in the treatment of malaria is based on the susceptibility of the genus plasmodium to free radicals and oxidants as well as the interference or inhibition of a metabolic synthesis pathway of a molecule essential to the parasite [4]. In fact, several substances used as anti-malarials such as chloroquine, primaquine and artemisinin are pro-oxidants, which is why they have pharmacological power. Quinine is one of the quinolines as chloroquine and primaquines hence it can exert pro-oxidant effect [5].

In recent years, several plant extracts and other natural products have been tested for their antioxidant properties. The mechanism of the disease is interfered by the substances by modulating the cellular signaling pathway and not by directly reducing the parasites to death [6]. Furthermore, the use of anti-oxidant supplements can reverse or minimize the oxidative damage to host caused by the free radicals released by the use of antimalarial drugs. For example, administration of curcumin, an herbal antioxidant obtained from *Curcuma longa*, prevented hepatotoxicity in rats treated with chloroquine [7]. Most of plants use for medicinal purposes have been correlated to their possession of antioxidant activity [8,9]. Antioxidants quench, scavenge and suppress the formation of reactive oxygen species and free radicals or oppose their actions [10].

Moringaoleifera is an economically important tree and vegetable and preliminary evidence suggest that it has an antioxidant and anti-inflammatory potency [6,11]. It contains compounds similar to sulforaphane and appears to be protective when orally ingested. *Moringaoleifera* is an herbal plant with immense medicinal value because of its antioxidant properties such as increased superoxide dismutase (SOD), increased catalase and reduced lipid peroxidation activities [12]. The plant is also a rich source of vitamins and antioxidants. It contains

good amount of proteins, minerals, vitamin A, vitamin B complex, essential amino acids and high content of vitamin E [13].

Moringaoleifera L (*Moringaceae*) known commonly as Ben oil tree or drumstick tree in English language, '*Okweoyibo*' in Igbo, '*Gawara*' or '*Habiwal*' in Hausa, '*Adagbamaloye*' or '*Ewe Igbale*' in Yoruba and *Morinka* in Ibibio grows rapidly in most regions and climatic conditions of Nigeria. It is an important food commodity which has had enormous attention as the 'natural nutrition of the tropics' [14]. A number of medicinal properties have been ascribed to various parts of this tree. Most parts of this plant: root, bark, gum, leaf, fruit (pods) flowers, seed and seed oil have been used in folk medicine in Africa and South Asia [15]. It has been used for the treatment of inflammation, infectious diseases, cardiovascular, gastrointestinal, and hematological and hepatorenal disorders [16,17].In view of the antioxidant properties of *Moringaoleifera* leaves, this study is aimed at examining its neuro-protective effect on the histomorphology of inferior colliculus of adult Albino Wistar rat following experimental administrations of quinine.

II. MATERIALS AND METHOD

2.1 Quinine and *Moringaoleifera*

Injectable quinine hypochloride was obtained from Buchler GmbH Germany. Appropriate conversion practice was used to calculate the therapeutic and experimental dosages determined per kilogram (kg) body weight of the animals. The injections were given intramuscularly on the thigh.The *Moringaoleifera* leaf samples of the same species were collected from a local farm in Uyo, AkwaIbom State, washed and transported under hygienic condition. The leaves were identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Uyo. *Moringaoleifera*leaf extract concentrate was prepared by ethanolic extraction using Percolation Method described by United States Pharmacopoeia Convention Inc (2000). The concentrated extract was preserved in refrigerator at the temperature of -4^{o}C until commencement of the research.

2.2 Acute Toxicity Test (LD_{50}) of *Moringaoleifera* Leaves Extract

The Acute toxicity testing (Lethal dose) LD_{50} of the extract of *Moringaoleifera* leaf was determined using modified Lorke's method (1983). The lethal dose of the extract was calculated to be 2500 mg/kg.10% and 20% of the LD_{50} value was used for the study.

2.3 Experimental Animals

Thirty-five (35) male Wistar rats weighing 180 - 200g were used in this study. They were obtained from the Animal House of the Faculty of Basic Medical Science University of Uyo, Uyo, Nigeria. The animals were acclimatized in the animal house of the Faculty of Basic Medical Sciences for two weeks during which they were fed with grower's mash manufactured by Pfizer Nigeria Ltd. Water was given *ad libitum*. The animals were randomly grouped into seven (7) groups, with 5 rats in each group. Permission and approval for the use and animal studies were obtained from the collage of health sciences animal ethics committee, University of Uyo, Uyo.

2.4 Treatment and Experimental Design

The administration of quinine and extract of *Moringaoleifera* leaf to the experimental groups started on the same day. The animals were treated based on the design below;

1. Control (No Treatment)
2. 10 mg/kg of quinine 8 hourly dailyfor7 days.
3. 10 mg/kg of quinine 8 hourly and 250 mg/kg of *Moringaoleifera*dailyfor7 days.
4. 10 mg/kg of quinine 8 hourly and 250mg/kg of *Moringaoleifera*dailyfor7 days followed by withdrawal of quinine and continuation of moringa for another 7 days.
5. 20 mg/kg of quinine 8 hourly dailyfor7 days.
6. 20 mg/kg of quinine 8 hourly and 500 mg/kg of *Moringaoleifera*dailyfor7 days.
7. 20 mg/kg of quinine 8 hourly and 500mg/kg of *Moringaoleifera*dailyfor7 days followed by withdrawal of quinine and continuation of moringa for another 7 days.

2.5 Collection of Sample

The animals were fasted twenty-four hours after the administration of the last dosage and then sacrificed under chloroform anaesthesia. Brain tissues were harvested and preserved in 10% buffered formalin for processing and staining for histomorphological changes using H and E technique.

2.6 Heamatoxylin and Eosin Technique

The whole brain tissues were fixed in 10% formal saline and then transferred to a graded series of alcohol. The tissues were dehydrated in 70% ethanol for 7 hours followed by 90% alcohol overnight and then in three changes of absolute alcohol for an hour each. The tissues were then cleared in xylene, infiltrated in molten paraffin wax in the oven at 58^{o}C. the tissues were then embedded in wax and blocked out. Serial sections of 5 microns thick were obtained from a solid block of tissues. The tissues were then stained with haematoxylin and eosin stains after which they were passed through ascending grades of alcohol, cleared in xylene and mounted in DPX mountant, allowed to dry at room temperature and observed histopathologically under digital light microscope [18].

III. RESULTS

The inferior colliculus from the Group 1 (control) to Group 7 animals are histologically illustrated in Plates I – 7 respectively. The inferior colliculus of control group showed normal histological features with the neurons appearing distinct with conspicuous cellular population (packed cell population). The neuronal and glial cells appeared normal with no vacuolation in the stroma of the section (Plate 1). The inferior colliculus of animals in the quinine treated groups (Groups 2 and 5) were affected, cellular degenerative changes such as hypertrophy and sparse cellular population were observed (Plates 2 and 5). Normal histological features were observed in the inferior colliculus of rats in Group 3 treated with 10 mg/kg of quinine and 250 mg/kg of *Moringaoleifera* (Plates 3). The inferior colliculus of rats in Group 6 were moderately affected showing cellular hypertrophy, sparse cellular population and focal vacuolation in the stroma (Plate 6). Seven days after withdrawal of quinine treatment, inferior colliculus of rats treated with quinine + *Moringaoleifera* in Group 7 showed no change in the normal histological features (Plate 7).

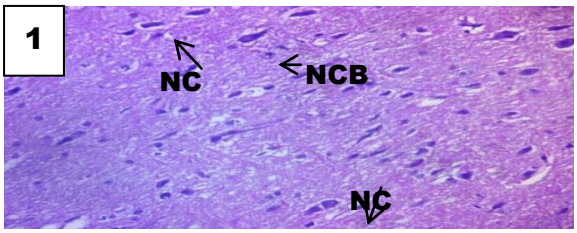

Plate 1: Photomicrograph of inferior colliculus of Group 1 rats without treatment revealed normal area of neuronal cell body (NCB), and neuronal density. The nerve fibers are not thickened. H&E method. Mag. X 400

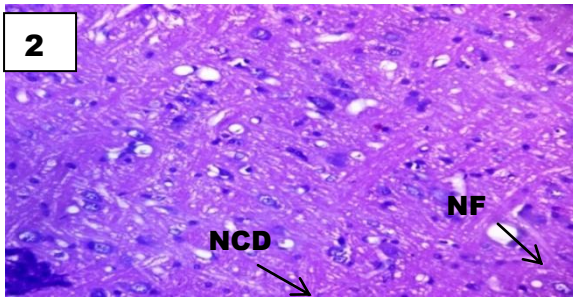

Plate 2: Photomicrograph of inferior colliculus of group 2 rats treated with 10 mg/kg body weight of quinine revealed neuronal cell degeneration (NCD**)**, and reduced neuronal density, thickened Nerve Fibers (NF). H&E method. Mag. X 400

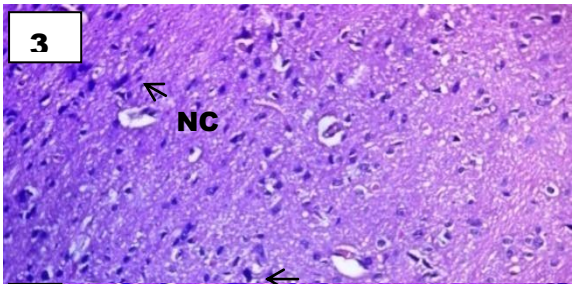

Plate 3: Photomicrograph of inferior colliculus of Group 3 rats treated with 10 mg/kg of quinine and 250 mg/kg of *Moringaoleifera* revealed normal neuronal cell (NC), and neuronal density, the nerve fibers are not thickened. H&E method. Mag X 400

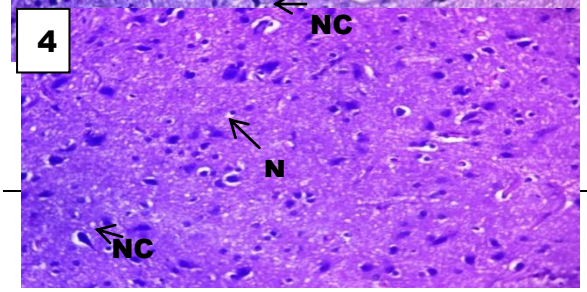

Plate 4: Photomicrograph of inferior colliculus of Group 4 rats treated with 10 mg/kg of quinine and 250 mg/kg of *Moringaoleifera* seven days after post-quinine treatment withdrawal revealed normal neuronal cell, and neuronal density, the nerve fibers texture restored. H&E method. Mag X 400

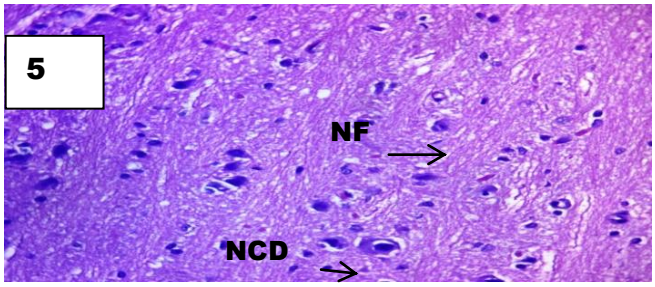

Plate 5: Photomicrograph of inferior colliculus of group 5 rats treated with 20 mg/kg of quinine revealed neuronal cell degeneration (NCD), thickened Nerve Fibers (NF) and reduced neuronal density. H&E method. Mag. X 400

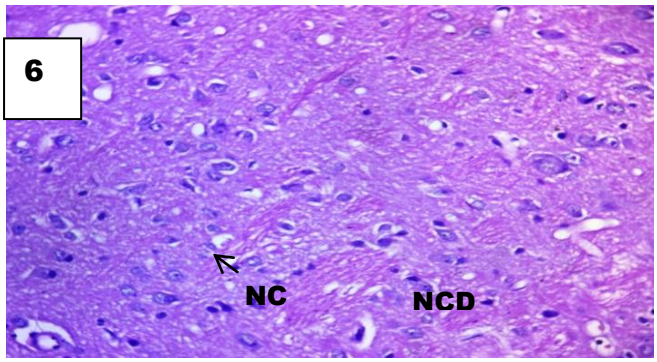

Plate 6: Photomicrograph of inferior colliculus of Group 6 rats treated with 20 mg/kg of quinine and 500 mg/kg body weight of *Moringaoleifera* leaf extract revealed slight neuronal cell degeneration (NCD), slight hypertrophy of neuronal cells (NC) and slightly thickened irregular nerve fibers and reduced neuronal density. H&E method. Mag X 400.

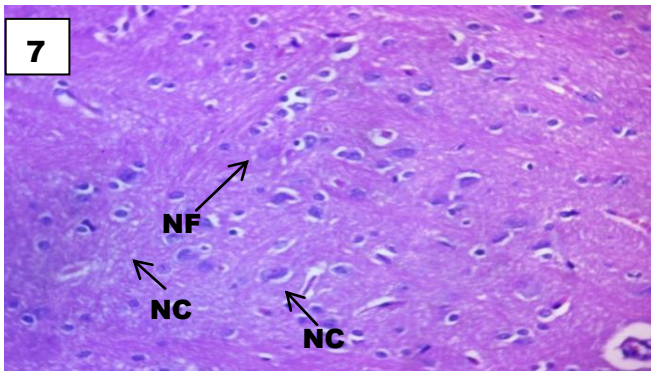

Plate 7: Photomicrograph of inferior colliculus of group 7 rats treated with 20 mg/kg of quinine and 500 mg/kg of *Moringaoleifera* seven days after post-quinine treatment withdrawal revealed normal neuronal cell (NC), Parallel Nerve Fibers (NF), H&E method. Mag X 400,

IV. DISCUSSION

The adverse effects of quinine on the cerebellum and inferior colliculus observed in this study may underlie the possible neurologic symptoms, such as tinnitus, as previously reported [19], following quinine treatment. Quinine has also been shown to cause damage to the nervous system (brain and spinal cord) of fetus, including damage to hearing, sense of balance, bleeding inside the eyes and other eye problems in animal studies [20]. A positive effect of *Moringaoleifera*on other brain tissues such as cerebral cortex has been reported [21]. The polyphenolic compound of *Moringaoleifera* leaf extracts might have played a vital role. It has been reported that the plant polyphenols provide protection against neurodegenerative changes [22]. Based on the effect of polyphenolic compound just mentioned, it was also possible that the neuroprotective effect of the plant extract was associated with these compounds.Plants have always played a major role in the treatment of human and diseases. Medicinal plants can be used in different form, as raw materials for the extraction of active compounds or for the extraction of abundant but inactive constituents which can be transformed by partial synthesis into active compounds, as drugs or extracts or traditional preparations. Medicinal Plants are therapeutics resource much used by the population of the world specifically for health care [23]. In our investigations, the effect of quinine and *Moringaoleifera* concurrent administration on the inferior colliculus of rats in group 3 treated with 10 mg/kg of quinine + 250 mg/kg of *Moringaoleifera*leaf extract revealed normal features. This is an indication that the structural damage that occur in the inferior colliculus of rats in Group 2 treated with 10 mg/kg of quinine only was absent in group 3 rats treated with 10 mg/kg of quinine + 250 mg/kg

of *Moringaoleifera* simultaneously. This points to the evidence that *Moringaoleifera*was able to restore the structural damage caused by low dose of quinine on the inferior colliculus to normal. *Moringaoleifera* anti-toxicity effect on cerebral cortex has been reported [24].*Moringaoleifera*leaf extracts exhibited the ability to provide neurite protection (protects against structural damage). This is reflected by preservation to or close to normal in terms of morphological structures as observed in group 3 rats. The presence of neurogenic agent such as *Moringaoleifera* leaf extracts could ameliorate the process by providing a positive stimulation while damping the neurodegenerative effect of quinine and prevent erratic uncoordinated stimulation. Our observation in groups 6 treated with 20 mg/kg of quinine + 500 mg/kg of *Moringaoleifera* simultaneously revealed that structural damage occurs on the inferior colliculus of rats in these group.Comparison between treated groups revealed that group 2 rats treated with 10 mg/kg of quinine and group 3 rats treated with 10 mg/kg of quinine + 250 mg/kg of *Moringaoleifera* revealed sections of rats' inferior colliculus with normal morphology in group 3 rats compared with group 2 rats which revealed degenerative changes. inferior colliculus of animals in group 5 treated with 20 mg/kg of quinine revealed a moderate degeneration compared with group 6 animals treated with 20 mg/kg of quinine + 500 mg/kg of *Moringaoleifera*which showed mild degenerative changes. These are indications of the neuroprotective effect of *Moringaoleifera*.The recovery of the spontaneous cases indicates the potential for the reversibility of the histopathological signs. This reversibility suggests that in natural cases, withdrawal of quinine treatment will result in recovery of the affected animals as observed in quinine low dose group treated with 10m/kg of quinine. This is confounded by the attempt of the central nervous system (CNS) precursor's cells to regenerate cerebellar and inferior colliculus tissues during post-quinine treatment withdrawal. Thus,*Moringaoleifera*provided the exogenous neurogenesis cue that drive recruitment and maintenance of neuronal cells.

V. CONCLUSION

This study has shown that administration of *Moringaoleifera* leaf extract protects against neurodegeneration and neuronal toxicity in albino Wistar rats due to quinine administration.

REFERENCES

[1] Halliwell B. Free Radicals Antioxidants and Human Diseases: Where are we now? *Journal of Laboratory Clinical Medicine*, 1992; 119: 598–620.

[2] Jenner B. Nervous Oxidative Damage. *Journal of Life Sciences*, 1996: 19(3): 204-209.

[3] Marcsinin SR, Jin X, Bettger T, Maculley N, Sousa JC, Shanks G, Tekwani BL, Sahu R, Richard GA, Sciotti RJ, Pybus BS. CYP450 Phenotyping and Metabolite Identification of Quinine by Accurate Mass UPLC Analysis: A Possible Metabolic Link to Black Fever. *Malaria Journal*, 2013; 12: 214-216.

[4] Grahame-Smith DG, Aronson JK. Tratado de FarmacologiaClinicaeFarmacoterapia. 3rd ed. *Guanabara Koogan: Rio deJaneiro*, Brazil, pp. 65-76, 2004.

[5] Zhang S, Chen H, Gerhard GS. Heme Synthesis Increases Artemisinin-induced Radical Formation and Cytotoxicity that can be Suppressed by Superoxide Scanvengers. *Journal of Cellular, Molecular and Biochemical Toxicology.* 2010; 180: 30-35.

[6] Ogbunugafor H, Igwo-Ezikpe U, Ozumba N, Aderekav S, Ugochukwu C, Onyekwelu O, Ekeehi A. In vitro and in vivo Evaluation of Antioxidant Properties of *Moringaoleifera*Ethanolic Leaves Extract and Effect on Serum Lipid Indices in Rats. *International Journal of Medical Sciences.* 2012; 5(4): 397-403.

[7] Daltani T. The Effect of *Curcuma longa* on Liver of Chloroquine Treated Rats. *Oxford Journal of Medical Sciences.* 2010; 30(9): 721-730.

[8] Middleton E Jr, Kandaswami C, Theoharides TC. The Effects of Plant Flavonoids on Mammalian cells: Implications for inflammation, Heart Disease Cancer. *Pharmacological Reviews*, 2000; 52: 673-751.

[9] Sofidiya MO, Odukoya OA, Familoni OB, Inya-Agha SI. Free Radical Scavenging Activity of some Nigerian Medicinal Plant Extracts. *PakistanJournal of Biological Sciences*, 2006; 9: 1438-1441.

[10] Adesegun SA, Elechi NA, Coker HAB. Antioxidant Activities of Methanolic Extract of *Sapiumelliticum*. *Pakistan Journal of Biological Sciences*, 2008; 11:453-457.

[11] Ilesanmi F, Akinloye O, Adeboye, Ilesanmi O. Comparative Evaluation of in vitro Antioxidant Properties of *CajanusCayan* seed and *Moringaoleifera*leaf extracts. *International Journal of Biochemical Research and Review* 2014; 4(2): 453-474.

[12] Ranira G, Rimi H, Kanshik R, Debajani G. Effect of *Moringaoleifera* in Experimental Model of Alzheimer's Disease: Role of antioxidants. *Annual* of *Neuroscience*, 2005; 12: 3.

[13] Das JM. (1965). Free Amino Acids and Carotennes in the Leaves of *Moringaoleifera* Lam. Synonym: Moringapterydosperma. *Current Science*, 1965; 34: 374-378.

[14] Anwar F, Bhanger MI. Analytical Characterization of *Moringaoleifera* Seed Oil Grown in Temperate Regions of Pakistan. *Journal of Agricultural and Food Chemistry*, 2007; 51: 6558– 6563.

[15] Fahey JW. *Moringaoleifera*: A Review of the Medicinal Evidence for its Nutritional, Therapeutic and Prophylactic Properties. Part 1. *Journal of Phytochemistry*, 2005; 47: 123-157.
[16] Morimitsu Y, Hayashi K, Nakagama Y, Horio F, Uchida K, Osawa T. Antiplatelet and Anticancer Isothiocyanates in Japanese Horseradish, Wasabi. *Biology Factors*, 2000; 13: 271-276.
[17] Siddhuraju P, Becker K. Antioxidant Properties of various Solvent Extracts of Total Phenolic Constituents from three different Agro-climatic Origins of Drumstick Tree (*Moringaoleifera*Lam). *Journal of Agricultural and Food Chemistry*, 2003; 15: 2144-2155.
[18] Cole EC. Studies on Hematoxylin Stains. *Stain Technology*, 1948; 18: 125-142.
[19] Manolette R. Adverse Effects of Quinine Treatment. *Journal of Biological Chemistry*, 2001; 12: 587-594.
[20] Macromedex RV. Neurotoxic Effect of Chloquinine. *Journal of Neuroscience*, 2001; 56: 257-287.
[21] Gupta RG, Kannan M, Sharma M, Flora SJ. Plants Natural Antioxidants. *National Proceedings Radiance*, 2005; 5: 326-334.
[22] Simonyi BT. Effects of Plants Polyphenols in Nervous Tissue. *Journal of Neuroscience*, 2005; 45: 578-597.
[23] Akpan E.J. Phytochemical and Nutraceuticals in Health Affair: The Biochemical Perspective, University of Uyo Press, Uyo, pp 10-15, 2014.
[24] Owolabi J, Williams F. Histological Assessment of *Moringaoleifera* Anti-Toxicity Actions Against Lead-Induced Damage to the Cerebellar Cortex in Animal Models. *International Journal of Moringa and Nutriceutical Research*, 2014: 1(1): 75-84.